Appendix

INTRODUCTION

This section of the book is devoted to methods. Included are descriptions of methods for rearing ticks, preserving ticks, preparing them for study, field sampling methods, design of field studies, and other procedures for studying ticks. Most of the methods described are those used in my laboratory or field stations.

LABORATORY ENVIRONMENT

Virtually any combination of rooms can be adapted for use in colonizing and studying ticks. Investigators with a small colony of a single species, or those who feed and house ticks intermittently, may use a corner of their laboratory for storing ticks, and any available work bench for periodic feeding on hosts. However, for those working with large colonies or many species, dedicated space and specialized facilities becomes necessary. When resources permit, a specialized facility should be planned. When constructing a new facility, the assistance of an architect is recommended to ensure the optimal use of space for the various activities involved in the tick colonization and study program. A simple tick-breeding facility is illustrated in Fig. A.1. If possible, one or more adjacent rooms should be made available for biological experiments, taxonomic study and administration.

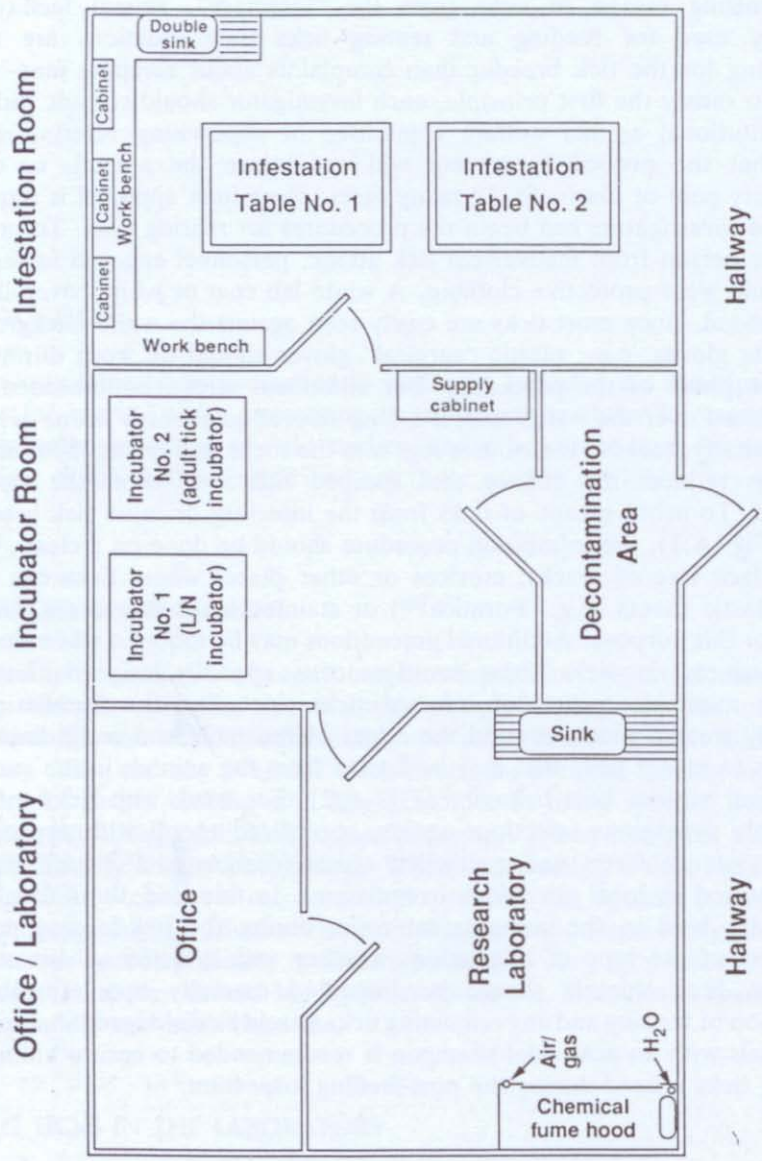
FEEDING TICKS IN THE LABORATORY

Ticks require animal blood for their development and reproduction. Although some species can be fed on whole blood via membranes (see below), most require an animal host for attachment and blood feeding. Methods vary among investigators and laboratories. Investigators usually adapt aspects of these methods to their specific environments, e.g., university laboratory, specialized

research facility, private laboratory, and so on. The following are descriptions of some of the more commonly used techniques. Cardinal principles for feeding ticks include (1) assuring humane care for laboratory animals; (2) taking precautions that minimize the risk of accidentally infesting the investigator, his or her technicians and/or students while feeding ticks; and (3) minimizing escape of ticks from the "insectary", animal facility, or laboratory used for feeding and rearing ticks (few situations are more embarrasing for the tick breeder than complaints about escaping man-biting ticks!). To satisfy the first principle, each investigator should consult with the local institutional animal welfare committee or supervising veterinarian to ensure that the proposed protocol will not injure the animal, or cause unnecessary pain or discomfit. In many cases, committee approval is required before the investigators can begin the procedures for rearing ticks. To protect his or her person from inadvertent tick attack, personnel engaged in feeding ticks should wear protective clothing. A white lab coat or white coveralls are recommended, since most ticks are easily seen against the white background. Disposable gloves, e.g., plastic "surgical" gloves should be worn during the infestation phase of the procedure. For additional safety, double-sided tape can be placed over the wrists and, if a long-sleeved garment is worn, over the cuffs. Similarly, tucking the trouser legs into the socks and taping them around one's legs reduces the chance that escaped ticks will enter the clothing unnoticed. To avoid escape of ticks from the insectary or other tick breeding facility (Fig. A.1), the infestation procedure should be done on a clean, white table surface free of cracks, crevices or other places where ticks can hide. Strong plastic sheets (e.g., Formica™) or stainless steel provides a suitable surface for this purpose. Additional precautions may be required when working with disease-carrying ticks. Some investigators use specially designed infestation tables to minimize escape of infected ticks, including the installation of chemically treated moats around the edges of the table and metal back and side walls to reflect ticks that may be hurled from the animals in the event of unexpected, violent host behavior (Fig. A.2). For work with ticks infected with highly contagious infectious agents, specialized rooms with appropriate air flow systems, filters and controlled access (designated P-3 facilities) are often required by local government regulations. In this case, the tick-infested animals are held in the containment room during the tick-feeding period. Regardless of the type of infestation, whether with infected or disease-free ticks, the host animals should be inspected carefully upon (presumed) termination of feeding and any remaining ticks should be dislodged. Shampooing the animals with an acaricidal shampoo is recommended to ensure killing any surviving ticks missed during the post-feeding inspection.

Ixodidae

Many ixodid species can be reared on common laboratory animals, e.g., mice, rats, guinea-pigs, and rabbits. Rearing methods are specific for each tick species, especially in the choice of hosts. Persons planning to rear ticks should consult with a specialist before proceeding with the rearing program. The


Double sink
Cabinet
Cabinet
Cabinet
Work bench
Work bench
Infestation Table No. 1
Infestation Table No. 2
Infestation Room
Hallway
Supply cabinet
Incubator No. 2 (adult tick incubator)
Incubator No. 1 (L/N incubator)
Incubator Room
Decontamination Area
Sink
Office / Laboratory
Office
Research Laboratory
Hallway
Air/ gas
H_2O
Chemical fume hood

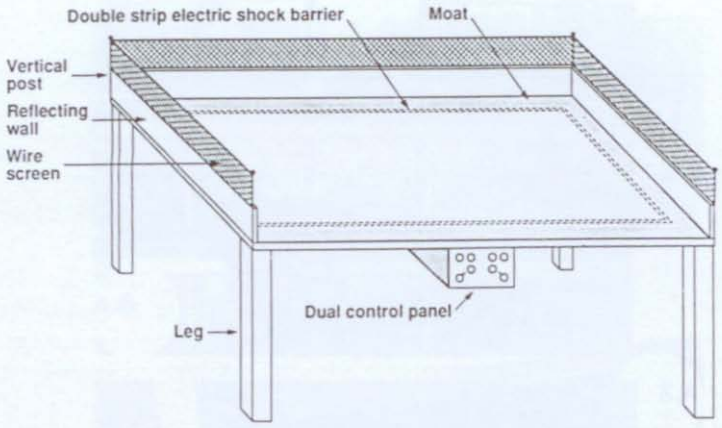
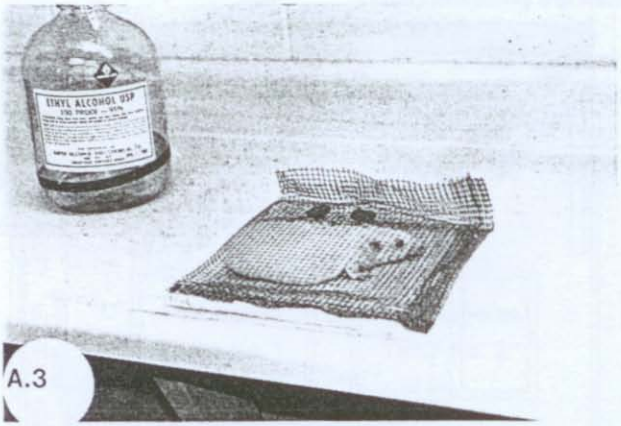


Figure A.2 Infestation table for minimizing escape of ticks during the infestation process. The table surface (smooth formica or similar plastic) is surrounded by an electric shock barrier (two adjacent strips of aluminum strips) activated by a control panel on the front of the table. The operator can adjust the current flow to provide a barely detectable, tingling sensation when touched by the human hand; ticks crossing the barrier are momentarily shocked and retreat. A moat filled with oil can be installed just outside the electric strips for additional security. A 15 cm high reflecting wall surrounds the table on three sides. This is needed in the event that tick-infested animals engage in violent behavior, projecting ticks in various directions. Drawers are omitted since they may provide hiding places for escaped ticks.

Figure A.1 (*opposite*) Diagrammatic representation of a simple tick-breeding facility with adjacent office and research laboratory. The facility consists of three parts: (1) the decontamination area; (2) the incubator room; and (3) the infestation room. The standards for ventilation, lighting, and room construction are those normally expected in a modern animal facility. Tick breeder personnel enter the facility via the decontamination area, change into regulation dress (e.g., white coveralls, lab coats, disposable boots, etc.) which are stored on racks or in cabinets in this area. A large bin should also be located in this area. Wearing proper dress, the tick breeder enters the incubator room or the infestation room. Feeding ticks is done in the infestation room where two specialized infestation tables are provided (see Fig. A.2). Work on the colony, e.g., counting ticks, washing vials, etc., is done in this room. Logs or other records may also be stored in this room. In addition to the ceiling flourescent ceiling lights) additional lighting should be provided over the work benches. The floors should be sealed with a good quality epoxy-based covering that extends approximately 15 cm onto the walls, providing a cleanable surface and eliminating cracks or crevices where escaped ticks may hide. Sticky tape should be used around doorways and doorsills to prevent escape of ticks from the breeding facility. A center floor drain, each fitted with a locking screen cover, should be included in each room to facilitate cleaning and prevent clogging with trash.



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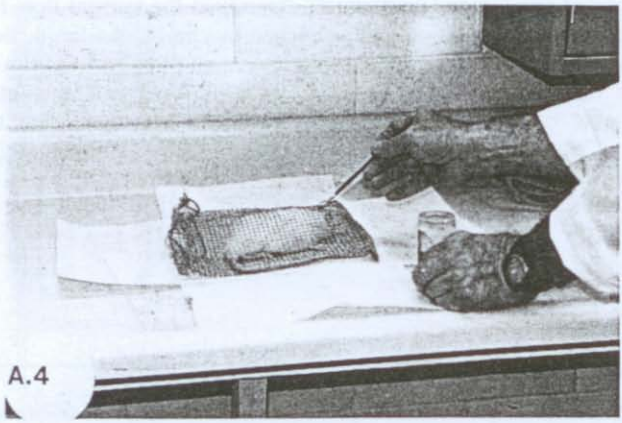

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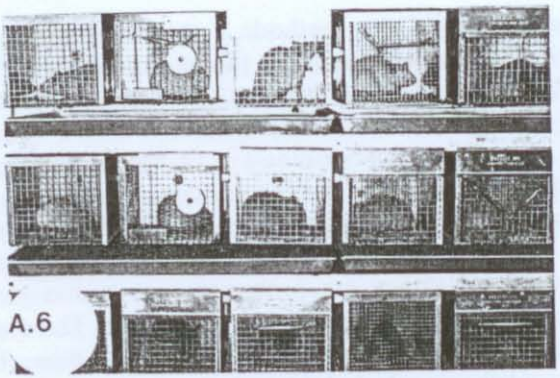
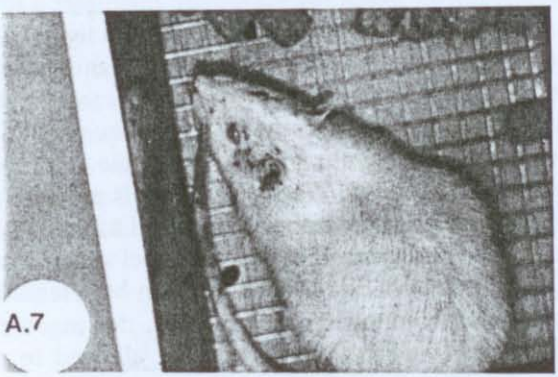
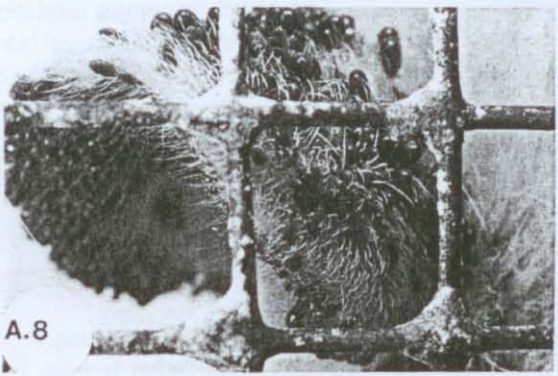






Figures A.3–A.8 Photographs illustrating techniques for feeding *Dermacentor variabilis* immatures on albino rats (*Rattus norvegicus*) and collecting fed ticks. **A.3.** Rats confined in funnel-shaped, wire screen (1/4 inch wire mesh) cages in preparation for infestation with larvae. One end of the funnel is opened, the rats are allowed to walk or run into the cage, and the end is closed by hand or with a pair of pliers. **A.4.** Technician infesting a caged rat with larvae, using a camel's hair brush. **A.5.** Following infestation, the paper towels are wrapped around the caged rat and secured with rubber bands. **A.6.** Tick-infested rats confined in hanging cages over waste trays covered with paper towels. Engorged larvae or nymphs drop from the host, collect on the paper towels, and are removed with an aspirator. Double-side tape around the edges of the tray prevents tick escape. **A.7.** Rat infested with feeding larvae. Note the engorging larvae on the ears and protruding from the fur around the head and face. **A.8.** Rat infested with feeding nymphs.

following is a description of the methods I use in my laboratory for the American dog tick, *Dermacentor variabilis* (Sonenshine, 1968b; Sonenshine et al., 1976b), the lone star tick, *Amblyomma americanum* and the deer tick, *Ixodes dammini*.

D. variabilis is reared on a combination of hosts. Rats, guinea-pigs or similar-sized small rodents are used for the immatures, while dogs, rabbits, or other convenient medium- to large-sized hosts are used for the adults. To feed the larvae, vials with thousands of individuals are exposed to daylight or intense artificial light while they are protected in a humid glass container. This is done to excite the larvae and terminate diapause. The exposure period is increased incrementally, e.g., 1 hour per day, for 3–5 days. This step may be omitted if the incubator in which the larvae are maintained provides intense illumination (e.g., 2000–3000 foot candles fluorescent light) and a timer to program the light:dark cycle. Otherwise, omission of this step may result in poor yields. Following illumination, the larvae are released onto the rats. To minimize grooming and enhance attachment, we confine each rat in a narrow, cylinder-shaped wire screen cage along with a small piece of fruit and one or two pellets of solid food (Fig. A.3). The wire screen is adjusted so the animal is squeezed gently, minimizing grooming behavior. Larvae are released onto the head and neck of each rat with the aid of a camel's hair brush (Fig. A.4). In my experience with this host, 500–1000 larvae can be released onto a single host without serious harm. Following their release, the caged rat is wrapped with paper towels secured with rubber bands and allowed to remain in this condition for 3–4 hours (Fig. A.5). The towel wrapping minimizes the escape of the ticks before they have had an opportunity to orient to the host and allows these parasites additional opportunities to crawl back onto the animal. Following the attachment period, the towel wrappings are removed. At this time, the technician may collect surviving unfed larvae remaining on the paper sheets with an aspirator (see below). The towels are decontaminated (see below) and discarded in a secure waste can. Next, the wire mesh cages are opened and the rats returned to their normal cages for housing during the feeding period. The ticks are allowed to feed while the animals are confined in their normal cages (Figs A.6–A.8).

To collect the engorged ticks after feeding, the tick-infested rats are held in hanging cages over waste trays covered with paper towels. Normal bedding may be used to trap urine and feces during the first 2–2.5 days after tick attachment, before larvae are able to complete engorgement and detach. Thereafter, the waste trays are covered with white paper towels for the remainder of the tick-feeding period to facilitate collection of engorged larvae. Tape, either double-sided tape or adhesive tape folded to expose a sticky surface, is placed around the edges of the waste tray to prevent tick escape. Ticks that detach from the rats fall through the grates of the hanging cages onto the paper towels. Although some ticks remain on the surface, contaminated by host feces, hair, and urine, most crawl under the edges of the paper sheets or onto the metal surface of the waste tray. To collect the ticks, the technician places the tip of the aspirator against the surface, activates the suction pump, and sucks the larvae into the collection chamber, avoiding fragments of food,

fecal pellets or other debri (Fig. A.9). Following collection of visible larvae on the surface, the paper is folded and larvae sheltering in the sheets below the top larvae are collected. This process is continued until the metal surface is exposed, from which the numerous larvae are easily removed. Larvae trapped on the tape can also be collected, but this must be done carefully with fine forceps to avoid puncturing their delicate bodies. If collections are made frequently, most larvae can be collected before they become trapped in the tape.

Many workers use water-filled trays instead of paper to trap engorge larvae. This method captures all of the detached ticks without the risk of injury when trapped on tape. However, it is much more labor intensive (in addition to being decidedly unpleasant). Some workers pour the contents through a large, paper-filled funnel, then spread the paper onto a large pan, allow it to dry and collect the larvae that crawl from the debris. When maximum containment is required, an apparatus similar to that described by Endris et al. (1986) may be useful (Fig. A.10).

In the case of *D. variabilis*, the same procedures used to feed and collect larvae are also used for the nymphs. However, great care must taken to avoid killing the small animal hosts due to exsanguination by the voracious nymphs.

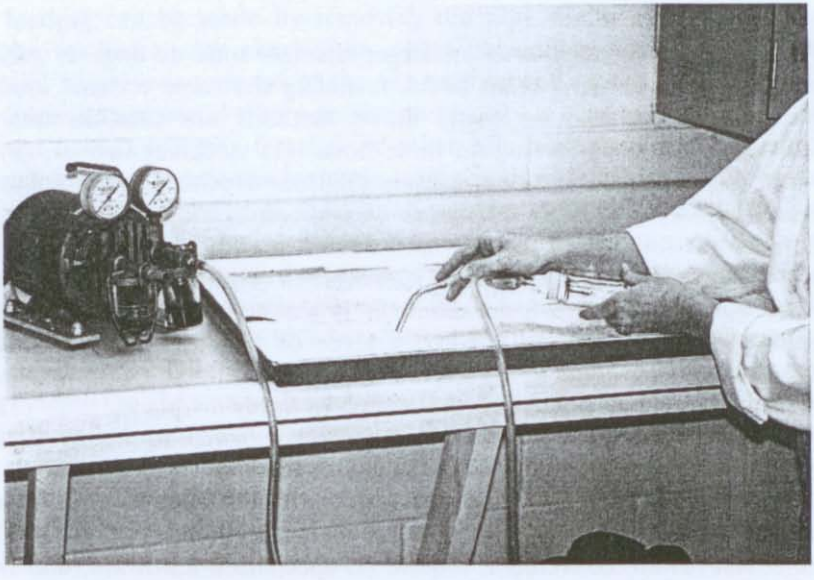


Figure A.9 Photograph showing technician collecting detached, fully engorged larvae from a paper-covered tray with an aspirator. The aspirator consists of a suction pump with adjustable gauges for controlling the vacuum, flexible tubing, a glass or plastic container with an inlet and an outlet port, and a flexible tube for sucking up the ticks. One piece of flexible tubing extends from the container to the inlet side of the vacuum pump; the other extends from the container and serves as the suction end of the aspirator.

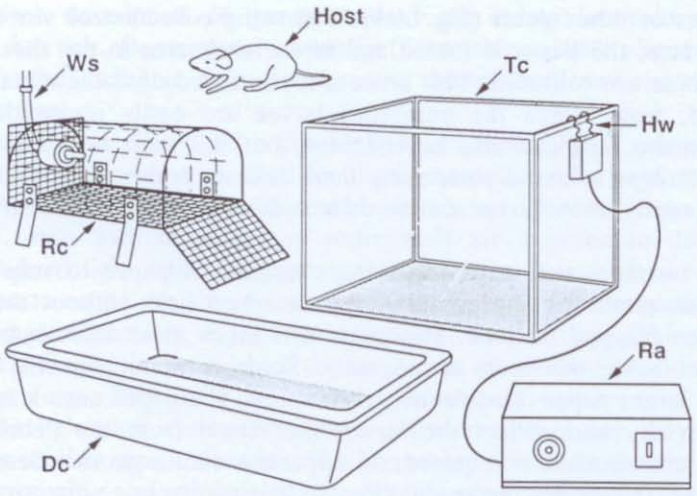


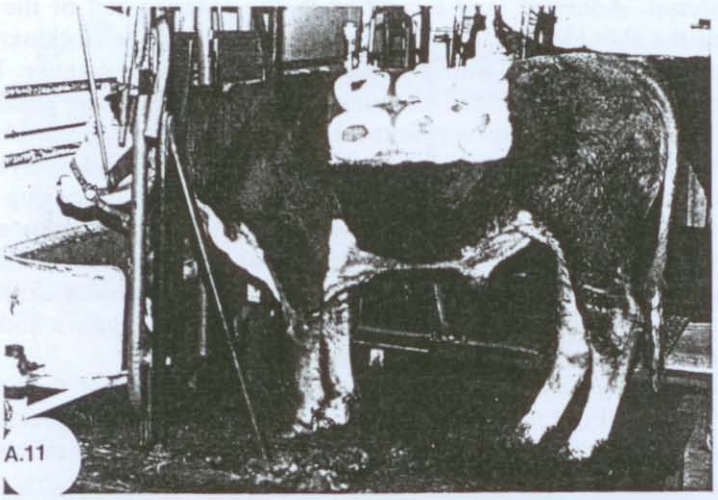
Figure A.10 Apparatus for feeding slow-feeding immature ticks (Ixodidae or Argasidae) on small animals. Food is supplied in the restraining cage ad libitum. Dc = Detergent container; Hw = hot wire; Ra = rheostat assembly; Rc = restraining cage; Tc = tick container; Ws = water supply. From Endris et al (1986), with permission from the Entomological Society of America.

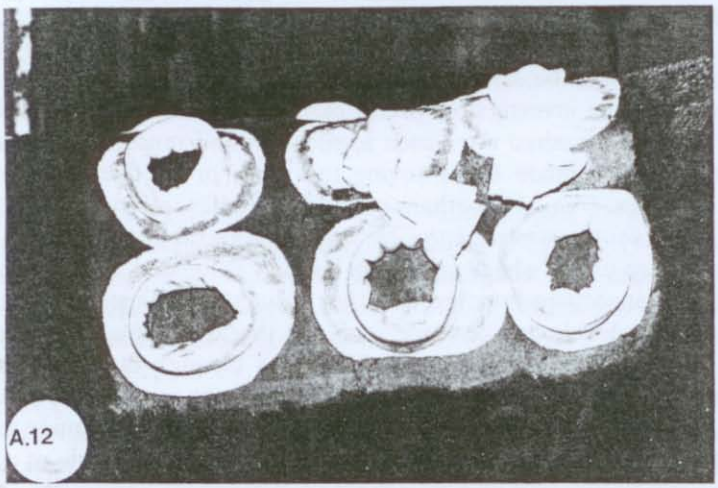
Adult ticks are best reared on larger animals such as dogs or rabbits, although a wide variety of other hosts, including the same rodents used for the immatures, can also be used. Three methods are used in different laboratories to facilitate feeding on these hosts: (1) stockinet sleeves; (2) ear bags; and (3) capsules. Stockinet sleeves (Balfour Health Care, Rockwood, Tennessee, USA) are tubes of coarse weave cotton cloth usually used as supports for plaster casts, bandages, and similar health care applications. For cattle or other large animals, the host is restrained (e.g., nose ring, head gate, etc.) and a circular area 5–8 cm diameter is shaved with an electric shaver (Oster Corp., Milwaukee, WI). Next, a piece of stockinet 7.5 cm diameter and *ca.* 15 cm long is glued to the shaved skin with a suitable adhesive cement. After the cement has dried and the sleeve is firmly in place, ticks can be released into the confined area. To minimize escape or risk to the investigator, it is best to insert the ticks in a vial. The vial is inverted inside the sleeve and held with one hand, while the cap is removed with the other hand. Following release of the ticks, the sleeve is twisted and sealed with tape (Figs A.11–A.13). Stockinet sleeves are convenient, require no specialized hardware, and rarely cause irritation or other adverse reaction to the host. Stockinet sleeves can also be applied to smaller animals such as rabbits (Figs A.14, A.15). In this case, a piece of stockinet tube 7.5 cm diameter × 10.0 cm long is prepared with holes near the front and back end for the legs. To minimize resistance by the host, we tranquilize the rabbits with PromAce™ (Acepromazine maleate) (Aveco, Fort Dodge, Iowa, USA). The section of stockinet is pulled over the animal's head and ears onto the midsection. The forelegs are inserted

into holes near the front end and the hindlegs into holes near the back end of the material. Adhesive tape is applied to the anterior end of the sleeve, just behind the shoulders. After ticks are released under the stockinet sleeve, the posterior end is sealed with adhesive tape in a similar manner. Ripping and tearing of the stockinet can be minimized by installing an "elizabethan" collar (so called because of its appearance) around the animal's neck. Although elizabethan collars work well, a more flexible, adjustable wide polyethylene collar has also proved effective in preventing rabbits from dislodging the ear bags (Watts et al., 1972). To examine the status of the ticks, the investigator need only remove the tape at either end, make his/her observations and replace the tape. Some investigators add (or substitute) a reusable cloth jacket, closed by buckles, straps, laces, or a zipper, around the animal's midsection. Similar systems can also be applied to dogs.

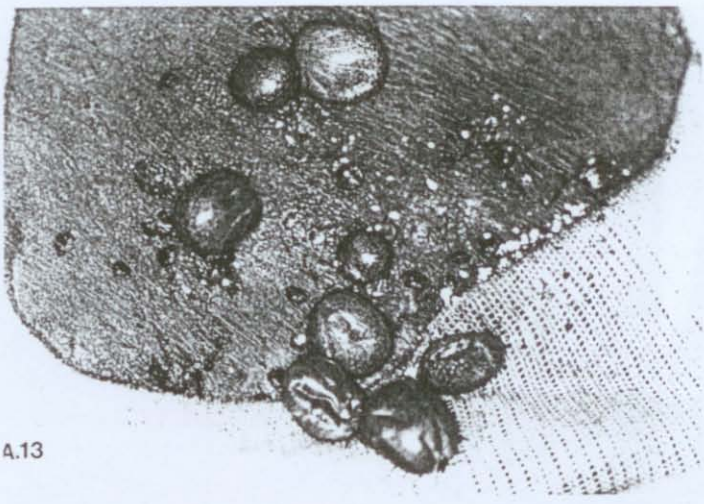
Ear bags are probably the most widely used method for feeding adult ticks on rabbits (Fig. A.16). Ear bags may be made from stockinet (see above), cotton cloth, or other suitable material. To prepare the rabbit for the ear bags, the fur around the base of the ears is removed with electric clippers. Next, the bags are inserted over the ears and glued to the shaved skin. Adhesive tape may be used to seal the bags and strengthen the enclosures. The opposite end can be sealed with tape after the ticks are released into the confined area. Some investigators prefer cloth bags closed by zippers, buttons, or laces, which are washed after each application and reused. Observations of tick feeding can be made by removing the tape, or by opening the zipper, buttons, or laces. An elizabethan collar or similar restraint is absolutely essential to prevent removal of the ear bags by the rabbits.

Capsules are useful where maximum security is needed, e.g., when disease infected ticks, engorging females, or other valuable specimens are being fed. An inexpensive capsule can be made from the threaded metal top of a mailing carton, i.e., cartons used to send small samples through the mail. The metal end is removed from the cardboard or other material forming the bulk of the tube and the latter discarded. Adhesive tape is applied to the inner and outer surfaces of the threaded metal base and taped to the shaved body of a suitable animal. Plastic bottles can also be adapted for a similar application; the neck and shoulder sections of the bottles are cut off and taped to the animal's body. After the ticks are released into the confined area, the metal or plastic cap is threaded into place. To examine the feeding ticks, the investigator need only unscrew the cap and observe the contents. In my laboratory, we fabricated capsules from acrylic plastic to provide a long-lasting supply of reusable capsules. Sections of a large tube (7 cm inside diameter) of 0.625 cm thick acrylic plastic were cut into suitable sizes to form the bases; a lathe was used to cut the threads on the outer margins. Sections of a larger tube (7.7 cm inside diameter) of the same thickness were cut into similar sizes to form the lids and threads were cut into the inner surfaces. Circular sections were cut from a flat sheet of plastic and glued to complete the lids (Fig. A.17). The bottom section can be buffed or tapered to avoid sharp edges. To avoid irritation of the rabbit's skin, adhesive tape is applied liberally around the bottom and sides, with the result that only this softer material is placed against





Figures A.11–A.13 Stockinet sleeves for confining feeding ticks on vertebrate hosts. **A.11**. Steer restrained by a head gate with stockinet sleeves cemented to the shaved skin of the flanks. Additional sleeves are secured on the opposite side of the animal. **A.12**. Enlargement of the same animal shown in the previous figure, showing details of the stockinet sleeves. Two of the sleeves in the top row have been infested with ticks. After releasing the ticks, the protruding end of each sleeve is tied into a knot. The sides of the remaining sleeves are folded and are ready to receive ticks. **A.13**. Enlargement of the interior of a stockinet sleeve showing feeding ticks (*Amblyomma americanum*) attached to the host skin. Engorged ticks that drop from the host remain in the sleeve until removed by the investigator, as shown in this photograph. To examine the infested area, the investigator merely unties the sleeves, rolls them back against the skin and observes the attached, feeding ticks. Photographs furnished courtesy of Dr J. Matthew Pound, Knipling Bushland US Livestock Insects Laboratory, USDA, Kerrville, Texas.



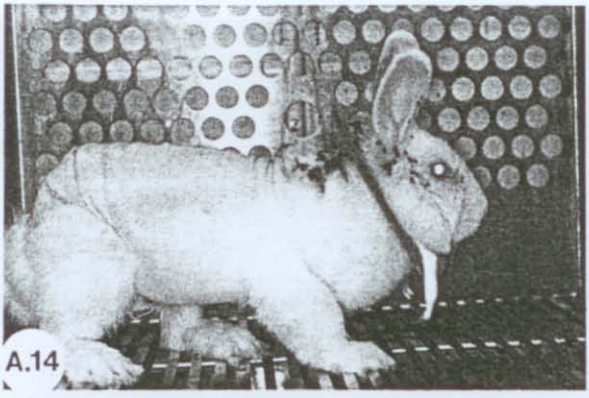
A.13

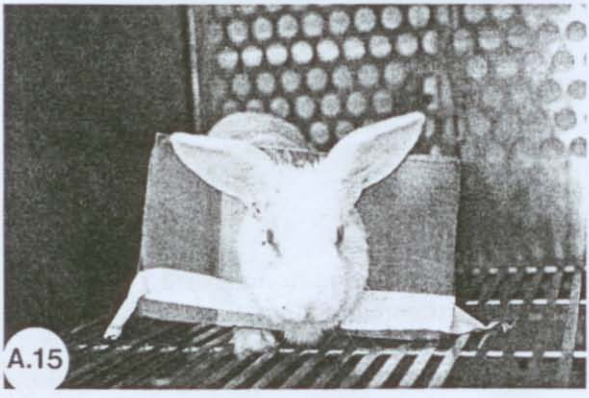
the animal's skin. Adhesive tape is used in the same manner as described above to attach the capsule to the midsection of a rabbit (Fig. A.18). Prior to installation, the rabbit is tranquilized (see above) and the midsection shaved with electric clippers. Following attachment of the capsule, the ticks are released and the lid screwed into place. An elizabethan collar is usually not necessary. Observations can be made directly through the clear plastic without disturbing the animal. For more detailed observations or removal of ticks, the lid is unscrewed. When installed correctly, these devices are well tolerated by the rabbits and there is no evidence of pain or discomfort. Ticks attach readily within the capsular area, where they remain confined (Fig. A.19). In my experience, these devices have proven to be the most secure and convenient means for feeding large numbers of adult ticks on rabbits. Unfortunately, improper installation may lead to sores, abscesses or other adverse skin reactions and require the capsule's early removal.

Feeding capsules can be modified in numerous ways to provide specialized feeding environments for unique applications. An example is that described by Apps et al. (1988) for collecting volatiles from ticks feeding on bovines (see Volume 1, Chapter 19). Another is that described by Wooten-Saadi et al. (1991), who developed a light-impermeable feeding chamber glued to the skin of cattle in order to study the effects of light exclusion on tick feeding and development (Fig. A.20).

Using the methods described above, thousands of *D. variabilis* adults can be reared per month. In a recent study on pheromones, we reared 14,313 females and a similar number of males over a 9 month period (Sonenshine et al., 1984).

A. americanum can be fed on a combination of avian and mammalian hosts. These ticks feed poorly on rats or mice and those hosts should be





Figures A.14, A.15 Protective mid-section stockinet sleeves for confining ticks on rabbits (*Oryctolagus cuniculus*) **A.14**. Horizontal view showing a New Zealand white rabbit with a stockinet sleeve around the midsection of the body. Note the holes for the fore- and hindlegs. An elizabethan collar was used to minimize disturbance of the device. Although most of the ticks released on the midsection of the body remained under the stockinet sleeve, some escaped and attached on the neck and head. **A.15**. Front view of a New Zealand white rabbit showing the elizabethan collar used to minimize grooming. The collar is secured around the neck with adhesive tape.

avoided. Instead, we use newly hatched chickens for feeding larvae (although rabbits have also proved satisfactory). To house the birds, cylindrical glass "mouse jars" or plastic cages designed for small rodents (e.g., Nalgene animal cages, 23.25 × 15.2 × 8 inches, Baxter, Columbia, Maryland, USA) are suitable. The bottoms of the cages are filled with litter to trap wastes. The top portions of the cages are removed and a ring of double-sided tape is placed around the uppermost edge to prevent tick escape. Infestation of larvae is done directly on the birds. To recover the engorged larvae, paper towels are substituted for the litter on the third day after infestation. The next day, the birds are removed (temporarily) and the engorged larvae collected with the aspirator. The process can be continued on successive days until all ticks have detached. Nymphal and adult ticks are fed on rabbits under stockinet sleeves in the same manner as described above for *D. variabilis*.

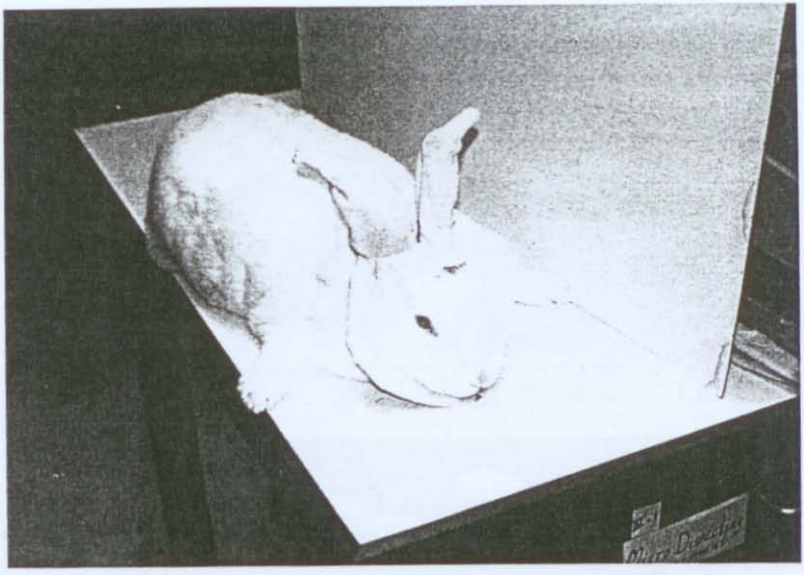


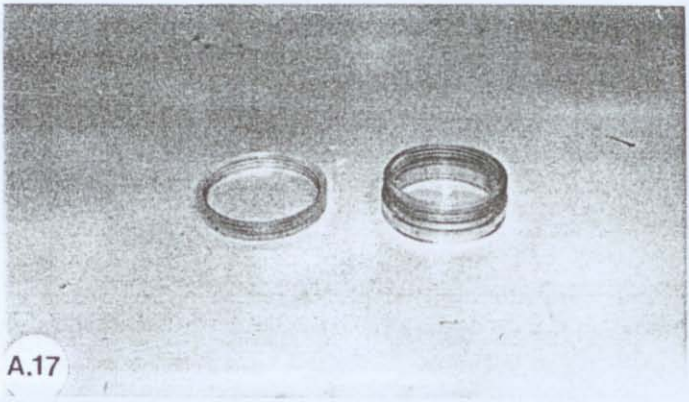
Figure A.16 A New Zealand white rabbit (*Oryctolagus cuniculus*) with ear bags and an elizabethan collar.

I. dammini can be fed on rabbits or a combination of small rodent and rabbit hosts. In my laboratory, we feed all life stages on rabbits when the purpose of the rearing effort is solely for colony production. For experiments such as vector competency trials (where *I. dammini* is the positive control), larvae are fed on white mice, hamsters, or gerbils. Hamsters are fitted with stockinet sleeves similar to that described for rabbits, but with 2.5 cm instead of the wider 7.5 cm. sleeves. Each animal is housed separately in a cage with a wire mesh floor placed on a glass or plastic tray. Paper towels are used to trap wastes and collect ticks. The edges of the waste tray are covered with double-sided tape to prevent tick escape, similar to that used with immatures of *D. variabilis* (Fig. A.9). Nymphal and adult ticks are usually fed on rabbits under stockinet or in capsules as described above.

Large animals are required for rearing some species of ticks, e.g., *Boophilus* spp. Restraints for large animals such as large domestic ruminants require special facilities usually found in barns, laboratories in a veterinary college or other animal buildings. A common restraint used with such animals is the stanchion, used to secure the head and neck. A discrete area of the body may be shaved, a stockinete sleeve glued to the shaved skin and ticks released within.

Argasidae

In contrast to ixodids, most argasid ticks feed rapidly (see Volume 1, Chapter 3). Consequently, the hosts are only confined for brief periods, e.g., 1–2 hours. To feed *Ornithodoros parkeri*, I release the ticks into a rectangular container filled with acid-cleaned sand, sawdust or other fine particulate



Figures A.17–A.19 Capsules used to confine feeding ticks on rabbits. **A.17**. Detail of disassembled capsule showing the base, the threaded lid; the tape strips used to fix the device to the animal are omitted. **A.18**. A capsule taped to the midsection of a rabbit. With this technique, the ticks remain completely confined within the capsule. **A.19**. Capsule removed to show how ticks attached within the confined space of the capsule. Most ticks attached near the edges of the device, forming a circle.

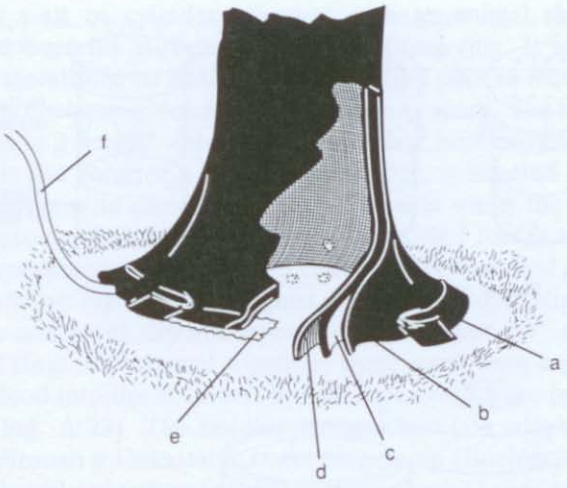
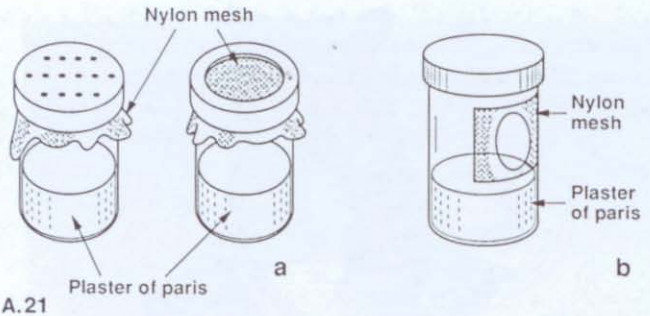


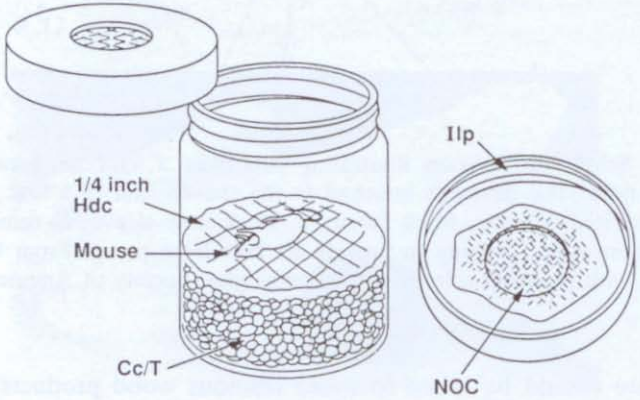
Figure A.20 Schematic diagram illustrating details of a light impermeable feeding chamber for ticks. The device is attached to the shaved skin of a host. **a**, Electrical tape; **b**, rubberized layer; **c**, black fabric; **d**, stockinette sleeve; **e**, cement to secure chamber to host skin; **f**, tubing to provide air flow from pump. From Wooten-Saadi et al. (1991), with permission from the Entomological Society of America.

material (care should be taken to avoid resinous wood products) where the ticks may burrow. Next, a rat previously tranquilized with PromAce™ is placed on top of the tick-infested container and a cover installed to reduce light (many argasid ticks are photophobic and feed best under dark conditions). Hungry ticks emerge from the sand or dust, attach indiscriminately to different areas of the rats body and feed rapidly. Most of the ticks complete feeding within one hour. After feeding, the swollen parasites drop from the host and burrow into the sand or sawdust where they also void coxal fluid (see Volume 1, Chapter 10). If desired for collection of information on molting, oviposition or other biological data, fed ticks can be recovered as they drop and placed in vials; however, care should be taken to provide absorbent surfaces for coxal fluid. This procedure for fast-feeding argasids is convenient, requiring little investment in cages or other equipment. Alternatively, (1) a rearing/feeding container may be constructed with a screen-covered lid or hole on the side which is attached tightly to an anesthetized small animal (e.g., mouse or hamster) with tape. Hungry ticks feed directly through the coarse mesh screen on the host body (Fig. A.21); or (2) a wide-mouth jar filled to a depth of 5–7 cm with absorbent particles (e.g., ground corn-cobs) covered by a wire-mesh screen may serve as the feeding container. Hungry ticks sheltering in the absorbent emerge to feed when a small animal, e.g., mouse or hamster, is placed in the jar as shown in the figure (Fig. A.22).

Avian parasites such as *Argas arboreus* can be fed on pigeons or similar birds. At NAMRU-3 in Cairo, Egypt, I observed a technician preparing the birds for this purpose by taping their feet and wings to the sides of the body



A.21



A.22

Figures A.21, A.22 Containers used for rearing/feeding nymphs and adults of fast-feeding argasid ticks on small animal hosts. **A.21**. Diagrams illustrating small containers that are taped to the body of small laboratory animal. A plaster of paris–charcoal mixture at the bottom of the container provides a moisture source for the incubating ticks. Ticks may feed through the nylon mesh when the lid is taped to the animal's skin. **a**, Individual rearing containers. **b**, Larger, group rearing container. **A.22**. Diagrams illustrating the construction of a large rearing/feeding container for fast-feeding small argasid ticks. Ticks sheltering in the corn-cob litter emerge in response to the small animal (e.g., mouse) placed on the wire screen above the litter material and attack the host. Cc/T = Corn cob litter; Hdc = hardware cloth; Ilp = inside of grooved lid; NOC = nylon/organdy cloth. From Endris et al. (1986), with permission from the Entomological Society of America.

and confining them individually in small containers. Ticks were introduced under the wings, allowed to feed, and the engorged specimens recovered from the floors of the containment boxes with an aspirator or with forceps.

Membrane Feeding

Fast feeding argasid ticks may also be fed through membranes on whole blood, blood plasma, or other solutions. Devices for this purpose generally have fresh

blood in small vials or cylinders covered with an animal skin or artificial membrane held over the surface with a tight-fitting ring. It is essential that all parts of the membrane be flush against the blood pool so that ticks inserting their mouthparts encounter blood rather than empty space. The blood container may be inserted in a heated water bath to simulate host body temperature. A cover, usually in the form of a blackened cylinder, is inserted over the lid of the circular membrane to provide a sheltered area in which the ticks may feed and facilitate recovery by the investigator. A modified feeder which I used in some of my experiments consists of a 3 cm diameter threaded metal chamber with a membrane on one side and a glass microscope cover slip on the other. The membrane and cover slip are separated by a rubber "O" ring. When the threaded metal rings are screwed together, these parts form a small chamber. To introduce blood into the chamber, hypodermic needles are inserted through the "O" ring (Fig. A.23). The needles are attached (via adapters) to flexible tubing passed through a Dekastaltic circulating pump (Buchler, Fort Lee, New Jersey, USA) to a blood reservoir held in a water bath (Lauer and Sonenshine, 1978). Among the advantages of this device are: (1) several feeders may be used simultaneously for different experimental treatments or different arthropod populations; and (2) constant hydrostatic pressure is maintained against the membranes of the feeders, avoiding air gaps and membrane collapse; in addition, circulating warm blood simulates host conditions more closely than a static blood pool.

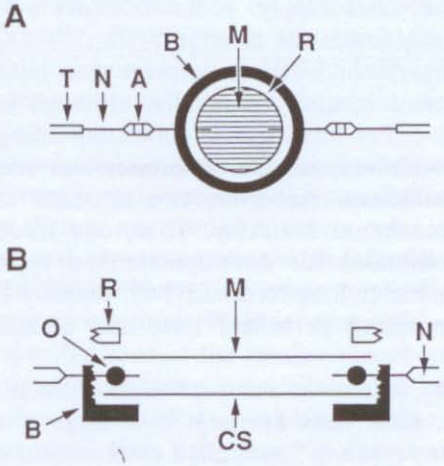


Figure A.23 Membrane chamber for fast-feeding ectoparasites, especially argasid ticks, through a membrane on blood or artificial media. **a**, Dorsal view; **b**, partially "exploded" cross-section. A = ♀ to ♀ adapter, B = base of chamber; CS = glass cover slip; M = membrane; N = 20 gauge hypodermic needle; O = rubber "O" ring; R = retaining ring; T = flexible tubing. From Lauer and Sonenshine (1978) with modifications; with permission from the Entomological Society of America.

INCUBATING TICKS FOR DEVELOPMENT AND REPRODUCTION

Following feeding, the engorged specimens must be placed under conditions that facilitate their development and reproduction. In most laboratories, incubators are used for this purpose although ticks will develop and reproduce even at room temperature when provided with adequate shelter and humidity.

Incubators are ideal for tick-breeding purposes because they provide a designated, controlled environment where conditions are uniform and optimized for each species (or life stage). Again, as with tick feeding discussed above, certain cardinal principles guide the selection of facilities by the tick breeder. Among the most important are: (1) a secure, protected cabinet, chamber, or room; (2) constant, adequate humidity; (3) constant temperature; (4) adequate ventillation; and (5) controlled lighting, i.e., constant light:dark (L:D) cycle.

The first principle is important to minimize the risk of ticks escaping into the laboratory where they may attack people or animals. Allowing an uncontrolled, wild infestation of ticks in the laboratory or animal facility creates an unacceptable hazard and reflects poorly on the tick breeder personnel. Thus, placing the ticks in a protected environment such as a locked cabinet or room is the first problem to be resolved. The remaining requirements noted are easily met by a suitable incubator. The simplest and least expensive commercial incubators only control temperature. For investigators planning to colonize a single species on a limited scale, a small table-top incubator may be sufficient. Humidity can be increased (but not truly controlled) by placing a large pan of water and a small electric fan inside the cabinet (on the bottom shelf); moist sand also works well because of the increased surface area provided for evaporation. Frequent inspections (e.g., once per day) should be made to maintain the water supply. A wet bulb/dry bulb indicator is useful for monitoring humidity inside the chamber.

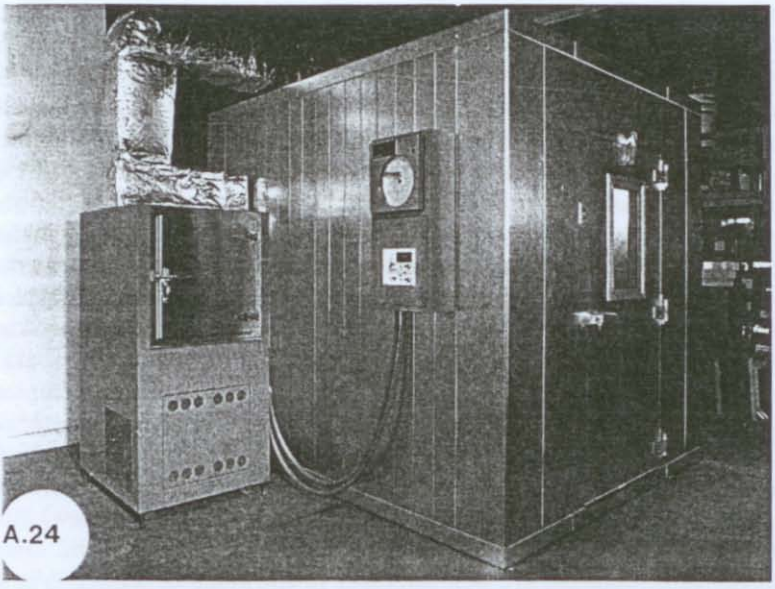
A commercial incubator with temperature and humidity control avoids the need to supplement humidity manually, although such instruments are usually more expensive. For investigators colonizing a single species or a small number of species with very similar environmental requirements, a single incubator may be sufficient. However, two or more temperature/humidity incubators provide maximum flexibility. Thus, one incubator may be set to provide optimum conditions for development and reproduction, while the other may be set to foster long-term survival, thereby extending the life of the colony and minimizing personnel time and animals required for tick feeding.

In my laboratory, where as many as eight different species have been colonized at a time, most ticks are kept in a large controlled environment room (Fig. A.24) or a "reach-in" controlled environment chamber (Fig. A.25). Technicians needing access are given the combinations to these facilities. Except when introducing or removing ticks, the room or cabinet is kept locked at all times. These types of chambers provide humidified air with a high rate of circulation (15 air changes per hour in the reach-in cabinet), facilitating rapid return of preprogrammed conditions. Although specifications vary, a well designed chamber can maintain temperature within 1°C and within 2–3%

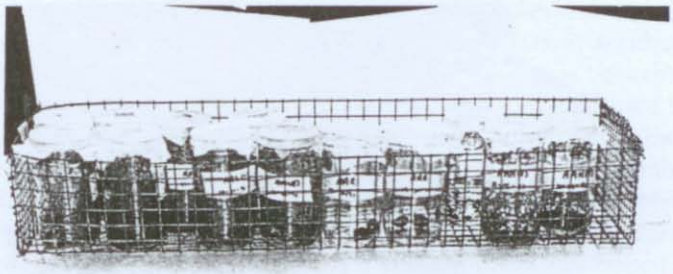
relative humidity. Changes in humidity can be made without changing the air temperature by altering the dew point of the water spray. An external control panel enables the investigator to adjust incubator conditions and monitor the values on a regular basis. Some manufacturers control chamber atmospheric moisture with water mist, and a humidity sensor; signals from the sensor circuit activate the humidifier. Others add an electric drier for rapid dehumidification. An external recorder, although not essential, is desirable to document incubator conditions for scientific data collection, instrument maintenance and quality assurance needs. Lighting in the incubator is usually in the form of low heat flourescent lamps. A 24-hour timer can be placed in the lighting circuit to enable the investigator to set the L:D cycle. Although some species, especially argasid ticks, can be incubated in total darkness, others are influenced by the length of the lighting period (see Chapters 23 and 24). Light intensity is also important. However, boosting the intensity of the incubator's internal lighting generates increased heat which is difficult to remove, leads to frequent failures and adds greatly to the system's expense. Within the incubator, ticks are stored in labeled vials (see below) held in removable containers. Normally, vials of similar material, i.e., the same life stage of the same species, are held together in the same container. Vials may be either plastic or glass and are caped with a perforated, gauze-covered lid. In my incubator, we use wire baskets to store groups of vials containing similar life stages (Figs A.26, A.27). Thus, all of the fed *D. variabilis* larvae are held in vials in one basket, fed nymphs in vials in another basket, unfed adults in another, and engorged, ovipositing females in yet another basket, all on the same shelf of the incubator. Each basket is labeled at the front for ready identification. Other incubator shelves are dedicated to other species. When colony size is small, several species may be stored on the same shelf, but in different baskets. Normally, conditions in this incubator are maintained at 27 ± 1°C, 92 ± 2% relative humidity and 16:8 hr L:D cycle. Unfed ticks not needed for experiments are transferred for long-term storage to a different incubator at a lower temperature.

A walk-in controlled environment room is recommended when colony size is expected to be very large, exceeding the capacity of the small reach-in chamber, where many shelves are needed to house colonies of many different species, where feeding and experimentation with infected ticks must be done in the same restricted environment, or other applications where a large controlled working area is essential. Walk-in controlled environment rooms must meet the same specifications as the smaller chambers.

Glass or plastic jars, desiccators or similar large containers provide a simpler, less expensive means for incubating ticks (Fig. A.28). Tick specimens confined in small vials are placed in the container along with a moisture source. Many workers use supersaturated solutions of specific salts to maintain a specific relative humidity within the container (Winston and Bates, 1960). Restoration of the specific humidity is dependent upon the size of the air space above the solution and the frequency with which the container is opened. At high humidities, mold is a common problem in such closed environments.



Figures A.24, A.25 Photographs of controlled environment chambers used to incubate and store ixodid ticks during their non-parasitic stages. **A.24**. Walk-in controlled environment room. **A.25**. Reach-in controlled environment chamber showing details of the shelves and containers for storing vials of ticks. Tick specimens are held in glass vials grouped in wire screen baskets; vials of the same type, e.g., engorged *D. variabilis* larvae are held in the same basket. All of the baskets of a given species are placed on the same shelf. Air flowing via large (15 cm diameter) ducts circulates between the chamber and the conditioner at the rear of the instrument at the rate of 15 air changes per hour. A "squirrel cage" type suction fan in the intake vent draws air from the humidifier over electric heaters, warming the air. Moisture is provided by a water



A.26

A.27

Figures A.26, A.27 Containers for maintaining fed ticks in incubators during their non-parasitic phases. **A.26**. Photograph showing a group of glass vials containing similar tick specimens held in wire screen baskets to be stored in the incubator for development or reproduction. The baskets serve as organizers to facilitate grouping of similar tick material for later use. **A.27**. Photograph of glass vials containing recently fed ticks. Note the gauze cover held in place by a perforated plastic "snap-cap" lid. Coded labels indicate the species, life stage and dates of drop off from the hosts.

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mist, generated by spraying water from a reservoir. The water spray is heated or cooled by a system of electric heaters and refrigeration coils. Sensors monitor air temperature in the tick chamber and in the water reservoir; opening and closing of their electrical circuits activates the heating and cooling units. A temperature control panel is located on one side of the instrument for convenient, visual display of information from the sensors and manual adjustment of the chamber conditions. Air temperature is controlled by heaters in the intake duct and refrigeration coils in the water reservoir. Relative humidity is controlled by adjusting the dew point, accomplished by cooling or heating the water used to generate the water spray as it enters the intake duct. The instrument is provided by Parameter Generation and Control, Black Mountain, North Carolina, USA. Figure A.24 provided courtesy of Parameter Generation and Control.

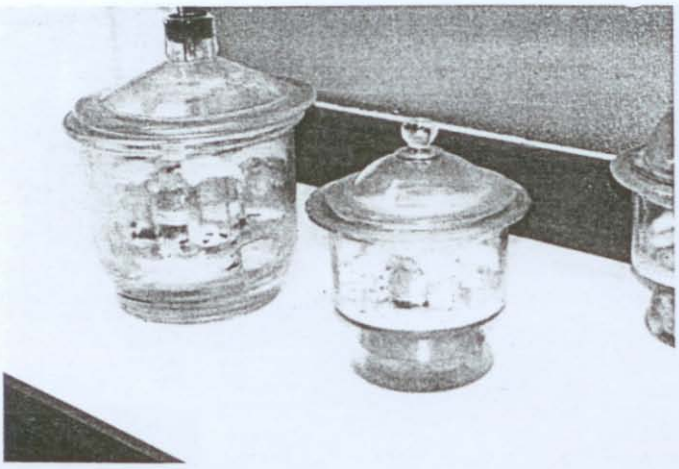

Figure A.28 Photograph showing small glass desiccators containing vials with large numbers of ovipositing *Ixodes dammini*. The desiccators are located in a walk-in controlled environment chamber (PGC, Black Mountain, North Carolina, USA). This chamber provides a secure environment in which ticks can be incubated under optimum conditions and used to infest hosts held in the same chamber for colony maintenance or for various types of experiments.

In addition to the chambers, rooms, or other large containers in which the fed or ovipositing ticks are stored, careful attention should be given to the small containers in which tick populations are held. The most commonly used procedure is to place the ticks in small glass or plastic vials. Thus, engorged larvae recovered after feeding are placed in small vials, often up to several hundred per vial, while smaller numbers of fed nymphs are placed in other vials. Care should be taken to avoid overcrowding. Frequently, investigators separate engorged females into individual vials, thereby ensuring that all of the progeny from a single female are easily distinguished. Vials should be labeled indicating the species and life stage; additional information that may be useful is the date recovered and the host. If a log is kept (see below), a simple coding system may be sufficient to identify the vials while more extensive data are recorded in the logbook.

For large facilities where tick-breeding programs are expected to furnish specimens for experiments and tests, a written (or computerized) log may be useful. Such logs can provide information on the number of individuals of each life stage, dates fed, expected molting dates, numbers of ovipositing females, and other data useful in making projections. Ready access to such information enables the tick breeder to respond to requests for supply of tick material and notify researchers regarding the date when needed specimens will be available.

Transporting ticks from the "insectary" area to research laboratories or the animal facility requires careful planning to avoid uneccessary accidents

that can contaminate crowded institutions and cause serious embarrassment. In agricultural environments such as an experiment station, accidental escape of ticks while they are being transported in the open spaces between buildings may initiate a "wild" infestation that can be difficult and expensive to contain. Within buildings, accidents are likely on stairways, in crowded hallways, sharp corners crowded with equipment, or similar locations where movement is obstructed. Ticks may be spilled from unprotected containers, poorly sealed vials, or broken glass vials. Where possible, ticks should be transported in secure containers, e.g., a small box which will not break if dropped and which will contain the ticks if the vials are broken or the vial covers dislodged.